OXFORD

International Journal of Neuropsychopharmacology (2020) 23(11): 738–750

doi:10.1093/ijnp/pyaa055
Advance Access Publication: 29 July 2020
Regular Research Article

REGULAR RESEARCH ARTICLE

Pro- and Anti-Inflammatory Properties of Interleukin (IL)6 in Vitro: Relevance for Major Depression and Human Hippocampal Neurogenesis

Alessandra Borsini, Maria Grazia Di Benedetto, Juliette Giacobbe, Carmine M. Pariante

Stress, Psychiatry and Immunology Laboratory, Institute of Psychiatry, Psychology and Neuroscience, Department of Psychological Medicine, King's College London, London, United Kingdom (Dr Borsini, Ms Di Benedetto, Ms Giacobbe, and Dr Pariante); Biological Psychiatry Unit, IRCCS Istituto Centro San Giovanni di Dio, Fatebenefratelli, Brescia, Italy (Ms Di Benedetto).

Correspondence: Alessandra Borsini, PhD, Stress, Psychiatry and Immunology Lab & Perinatal Psychiatry, Institute of Psychiatry, Psychology and Neuroscience, King's College London, G.32.01, The Maurice Wohl Clinical Neuroscience Institute, Cutcombe Road, London, SE5 9RT United Kingdom (alessandra.borsini@kcl.ac.uk).

Abstract

Background: Although the pro-inflammatory cytokine interleukin (IL)6 has been generally regarded as "depressogenic," recent research has started to question this assumption in light of the fact that this cytokine can also have anti-inflammatory properties. This bimodal action seems to be dependent on its concentration levels and on the concomitant presence of other pro-inflammatory cytokines.
Methods: We exposed a human hippocampal progenitor cell line, HPC0A07/03C, to cytokine levels described in depressed patients (IL6 5 pg/mL with IL1β 10 pg/mL or Macrophage Migration Inhibitory Factor (300 pg/mL) in healthy individuals (IL6 with IL1β, 1 pg/mL or Macrophage Migration Inhibitory Factor 10 pg/mL), as well as to the potentially anti-inflammatory, much higher concentrations of IL6 (50 000 pg/mL).
Results: Treatment with high concentrations of IL6 with IL1β or Macrophage Migration Inhibitory Factor (resembling depressed patients) decreases neurogenesis compared with low concentrations of the same cytokines (healthy individuals) and that this is mediated via production of, respectively, IL8 and IL1β in cell supernatant. Instead, treatment with very high, anti-inflammatory concentration of IL6 (50 000 pg/mL) together with high IL1β or Macrophage Migration Inhibitory Factor prevents decrease in neurogenesis and reduces both IL8 and IL1β. When high concentrations of both IL1β and Macrophage Migration Inhibitory Factor were used in co-treatment, as a model of treatment-resistant depression, we also demonstrated a reduction in neurogenesis and that this is mediated via a decrease in IL4; moreover, co-treatment with high IL1β and Macrophage Migration Inhibitory Factor and the very high concentration of IL6 prevented the reduction in neurogenesis and increased IL4.
Conclusions: Our results demonstrate that IL6 can exert both pro- and anti-inflammatory (potentially antidepressant) properties, depending on its concentrations and combinations with other inflammatory cytokines.

Key Words: interleukin-6 (IL6), interleukin-1beta (IL1β), macrophage migration inhibitory factor (MIF), neurogenesis, depression

Received: May 20, 2020; **Revised:** July 1, 2020; **Accepted:** July 21, 2020

Significance Statement

Several studies have shown that major depression is characterized by an increase in the production of IL6 and other inflammatory cytokines, but the prevailing model that IL6 is "depressogenic" has recently been put into question in light of the fact that this cytokine can also exert anti-inflammatory properties. In this study, we demonstrate for the first time, to our knowledge, that treatment of human hippocampal progenitors with very high concentrations of IL6 prevent reduction in neurogenesis caused by high concentrations of IL6 and IL1β or MIF (resembling depressed patients) and by high concentrations of both IL1β and MIF (resembling treatment-resistant depressed patients) via regulation of distinct signaling molecules, including increases in IL8 and IL1β and a reduction in IL4. Overall, our findings show the ability for IL6 to exert both pro- and anti-inflammatory as well as antidepressant properties, which are dependent on its concentration and the various combinations with other inflammatory cytokines.

Introduction

Several studies have shown that major depression is characterized by an increase in the production of interleukin (IL)6 and other inflammatory cytokines, including IL-1β, and tumor necrosis factor (TNF)-α (Dowlati et al., 2010; Osimo et al., 2020), but the prevailing model that IL6 is "depressogenic" has recently been put into question (Del Giudice and Gangestad, 2018). These findings have fundamental clinical importance as cytokines can directly contribute to the development of depressive symptomatology (Raison and Miller, 2013). These inflammatory proteins can induce stress-related neuroendocrine alterations and central neurotransmitter and neuroplasticity changes, reminiscent of those commonly seen in depression (Miller and Raison, 2016), and are elevated also during immunotherapy with interferon (IFN)-α, which can precipitate clinical depression (Capuron et al., 2003, 2007; Hepgul et al., 2016, 2018). In addition, higher baseline concentrations of 2 inflammatory markers, IL1β and Macrophage Migration Inhibitory Factor (MIF), can accurately predict lack of antidepressant response in depressed patients (Cattaneo et al., 2013, 2016), therefore confirming the fundamental role of cytokines for both the pathogenesis and treatment of the depressive disorder.

As mentioned above, recent evidence suggests that IL6 may not have only pro-inflammatory and "depressogenic" disease-promoting inflammatory effects but also exert anti-inflammatory properties (Raison et al., 2018). This bimodal action seems to be dependent on its concentration levels and on the concomitant presence of other pro-inflammatory cytokines (Pedersen and Febbraio, 2008; Felger and Lotrich, 2013). Indeed, relatively high concentrations of IL6, similar to those detected in blood and cerebral spinal fluid (CSF) of depressed patients, are considered detrimental, whereas even higher concentrations, sometimes thousands-fold higher than baseline levels—like those found in healthy individuals, mice exposed to fasting and exercise, or depressed patients treated with hyperthermia—are associated with anti-depressants or mood-elevating outcomes (Raison et al., 2018). Similarly, ketamine, which is able to produce a rapid antidepressant effect, also acutely increases peripheral circulating levels of IL6 (Park et al., 2016). However, while IL6 production is very high in individuals exposed to exercise or hyperthermia, levels of both IL1β and TNF-α remain in the "inflammatory" range (Raison, 2017), therefore suggesting that different cytokines may have differential effects in the context of depression.

As mentioned above, higher concentrations of peripheral cytokines, including IL6, are often correlated with higher levels in the CSF (Lindqvist et al., 2009; Tsuboi et al., 2018). Moreover, peripheral cytokines can penetrate the blood-brain barrier from the periphery and directly affect brain pathways underlying the depressive psychopathology (Miller and Raison, 2016). Neurogenesis is regarded as one of the major mechanisms potentially involved in depression as well as a fundamental process required for antidepressant efficacy (Santarelli et al., 2003; Boldrini et al., 2009, 2014). Indeed, using an in vitro model of human hippocampal neurogenesis, we have previously demonstrated the ability of IL6, IL1β, and IFN-α to cause reduced neuronal cell proliferation and neurogenesis, and of the 2 antidepressants, sertraline and venlafaxine, to prevent such inflammation-induced neurogenic changes (Borsini et al., 2017, 2018). Further work on our established model of depression in a dish has shown that these human neuronal precursors consistently respond to depressogenic challenges, like cortisol and inflammation, with a reduction in neurogenesis that is rescued by antidepressant strategies like antidepressants, anti-inflammatories, and omega-3 (Anacker et al., 2011, 2013a, 2013b; Zunszain et al., 2012; Horowitz et al., 2014; Borsini et al., 2017, 2019).

Although there is a good amount of evidence suggesting a negative role for IL6 on cell proliferation and gliogenesis (Borsini et al., 2015), to our knowledge, no one so far has investigated whether higher, potentially anti-inflammatory concentrations of IL6 could instead be beneficial for neuronal formation and how this phenomenon could be influenced by the combination of IL6 with other pro-inflammatory cytokines. Here, using our in vitro model of human hippocampal progenitor cells, we investigate whether treatment of cells with high concentrations of IL6 together with IL1β or MIF, resembling depressed patients, can detrimentally affect neurogenesis, and whether treatment with a much higher concentration of IL6, resembling the anti-inflammatory conditions, can instead prevent such changes. We then test whether treatment with both IL1β and MIF, as we have previously shown in our research on treatment-resistant depressed patients, can also affect neurogenesis, and whether treatment with the same very high concentration of IL6 can prevent such changes, also compared with treatment with the 2 aforementioned antidepressants, sertraline and venlafaxine (Borsini et al., 2017). Finally, we explore the mechanisms underlying this putative bimodal action of IL6 using selective antibodies against cytokines produced on exposure of cells to the various "depressogenic" conditions.

Methodology

Cell Culture—Multipotent human hippocampal progenitor cell line HPC0A07/03C (provided by ReNeuron, Surrey, UK) was used (Anacker et al., 2011, 2013a, 2013b; Zunszain et al., 2012; Horowitz et al., 2014; Borsini et al., 2017). This model was previously validated using a hippocampal newborn neuron specific marker, Prospero homeobox protein 1 (Prox1) (Anacker et al., 2013a). Cells were left to proliferate in the presence of

growth factors epidermal growth factor (EGF), basic fibroblast growth factor, and 4-hydroxytamoxifen (4-OHT). Differentiation was initiated by removal of the growth factors and 4-OHT. Detailed information on this cell line can be found in our previous publication (Anacker et al., 2013a).

In Vitro Treatment With Cytokines—Across the experiment described below, we have used a range of concentration of cytokines based on the existing literature. Specifically, we used low and high concentrations of IL1β (1, 5 pg/mL), TNF-α (1, 10 pg/mL), MIF (10, 300 pg/mL), and IL6 (1, 5 pg/mL), as found in blood and CSF of, respectively, healthy individuals (Lindqvist et al., 2009; Pawlitzki et al., 2018) and depressed patients (Piletz et al., 2009; Hestad et al., 2016; Kranaster et al., 2018; Tsuboi et al., 2018). Moreover, being guided by concentrations found in blood and CSF of healthy individuals or depressed patients exposed to interventions resembling anti-inflammatory conditions (Raison et al., 2018), as well as by a dose-response curve performed on our cellular model, we selected IL6 50 000 pg/mL as the most representative "anti-inflammatory" concentration of IL6. Indeed, our dose-response curve (IL6 1 pg/mL to 50 000 pg/mL) showed a U-shaped curve for neurogenesis (MAP2), that is, no changes with 1, 5, and 50 000 pg/mL and a significant decrease with the intermediate concentrations of 50, 500, and 5000 pg/mL (see supplementary Figure 1). Thus, for our experiments in co-incubation with other cytokines, we use the low, high, and very high concentrations of IL6 that alone do not affect neurogenesis. Of note, IL6Receptor is expressed on our cells especially during the differentiation period, that is, the time point during which all the investigations were performed (Johansson et al., 2008). Finally, IL4 (3, 30 pg/mL) was used in additional experiments, as production of this cytokine was reduced by treatment with high concentrations of both IL1β and MIF, while treatment with the very high concentration of IL6 (50000 pg/mL) together with IL1β and MIF increased the production of IL4 up to these concentrations in cell supernatant.

Differentiation Assays—To assess changes in neuronal differentiation, HPC0A07/03C cells were plated into clear 96-well plates (Nunclon) at a density of 1.5×10^4 cells per well. At least 6 independent experiments were conducted on independent biological cultures (i.e., originating from completely independent experiments in different days and from different cells passages), and each sample was tested in quadruplicate. After 24 hours, cells were cultured for 3 days in the presence of EGF, basic fibroblast growth factor, 4-OHT, and with low and high concentrations of IL1β (1, 5 pg/mL), TNF-α (1, 10 pg/mL), and MIF (10, 300 pg/mL). In particular, cells were treated with each individual cytokine either alone or in combination with low, high, and very high concentrations of IL6 (1, 5, and 50000 pg/mL). Treatment with selective antibodies—IL8 antibody (A) (0.5 µg/mL), IL1βA (0.1 µg/mL), IL6A (0.1 µg/mL), or sertraline (1 µM), venlafaxine (1 µM), or additional cytokines, IL4 (3 and 30 pg/mL)—was then added to some of the above experimental conditions with low and high concentrations of IL1β, MIF, and IL6 (see Figure 5; supplementary Figures 5, 7, and 8). After this initial proliferation phase, cells were washed and cultured in media containing the same cytokines/compounds in the same combination described above but without growth factors or 4-OHT for 7 subsequent days. This paradigm was used for all experiments. Finally, cells were rinsed with warm phosphate buffered saline and fixed with 4% paraformaldehyde for 20 minutes at room temperature. Detailed information on the differentiation assay can be found in our previous publication (Anacker et al., 2013a).

Immunocytochemistry—Differentiation into immature and mature neurons was assessed, respectively, with doublecortin (DCX) (Alexa 488 donkey anti-rabbit; 1:1000) and microtubule-associated protein 2 (Map2) (Alexa donkey 555 anti-mouse, 1:1000, Invitrogen). Apoptotic cells were examined using caspase 3 (CC3) (Alexa 488 donkey anti-rabbit; 1:1000; Invitrogen). 4',6-diamidino-2-phenylindole dye was used to label all cells. Detailed information on the immunocytochemistry procedure can be found in our previous publication (Borsini et al., 2018).

Automated Quantification of Immunofluorescence—The percentage of DCX, Map2, and CC3-positive cells over total 4',6-diamidino-2-phenylindole-positive cells was counted using an insight

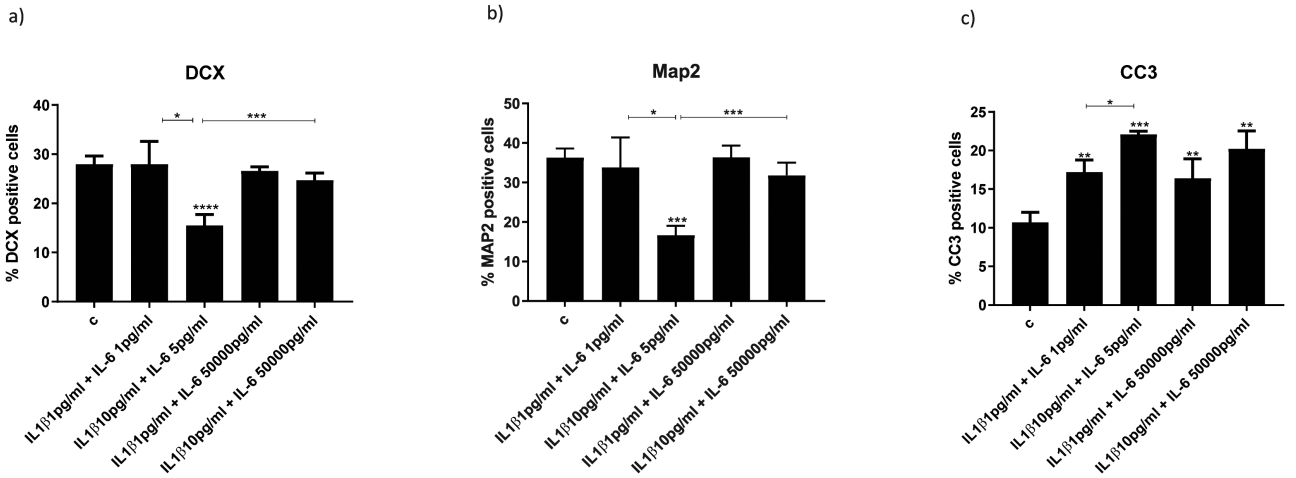

Figure 1. Very high concentrations of interleukin 6 (IL6) prevent interleukin 1beta (IL1β)- and IL6-induced reduction in neurogenesis. Treatment for 3 days during proliferation followed by 7 days during differentiation with a high concentration of IL1β (10 pg/mL) and a very high concentration of IL6 (50000 pg/mL) was able to prevent the decrease in both doublecortin+ and microtubule-associated protein 2+ cells caused by high concentrations of IL1β (10 pg/mL) and IL6 (5 pg/mL) compared with low concentrations IL1β and IL6 (1 pg/mL, both cytokines) (a, b). Co-treatment of cells with high concentrations of IL1β and IL6 increased the percentage of caspase 3 (CC3)+ cells compared with co-treatment with low concentrations of IL1β and IL6. However, co-treatment with IL1β and a very high concentration of IL6 did not prevent the increase in CC3+cells (c). One-way ANOVA with Bonferroni's post hoc test. Data are shown as mean ± SEM; $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, $^{****}P < .0001$, compared with vehicle treatment or as indicated.

automated imaging platform (CellInsight NXT High Content Screening Platform—ThermoScientific). Detailed information on the imaging analyses procedure can be found in our previous publication (Borsini et al., 2018). Due to the use of the automated cellular quantification platform, we obtained small differences in the percentage of DCX, Map2, and CC3-stained cells in the control condition compared with our previous publication, where manual counting was used (Anacker et al., 2011). See supplementary Figure 2 for representative images.

Multiplex Cytokine Measurement—Cell supernatants of differentiated cells were run on the Human ProInflammatory Singleplex and Multileplex Very-Sensitive Kit from Meso Scale Discovery (Gaithersburg, MD) according to the manufacturer's instructions. Briefly, 50 µL of prepared samples was added into each well of the Meso Scale Discovery plate, which was subsequently incubated for 2 hours with vigorous shaking at 1000 rpm at room temperature. The plate was then washed 3 times with 150 µL/well of wash buffer and 25 µL of detection antibody solution was added to each well followed by another 2 hours of incubation with vigorous shaking at 1000 rpm at room temperature. Finally, the plate was washed for 3 times with 150 µL/well of wash buffer, and 150 µL of 2× read buffer T was added to each well. The plate was analyzed in the SECTOR Imager machine for the measurement of IL1β, IL2, IL4, IL6, IL8, IL10, IL12, IL13, MIF, IFN-γ, and TNF-α.

Statistical Analysis—All statistical analyses were performed with GraphPad Prism 8.00. In particular, one-way ANOVA with Bonferroni's post hoc test is used for multiple comparisons across different cytokine treatment groups, whereas factorial ANOVA was used for multiple comparisons across different cytokine groups in the presence of antidepressants or cytokine antibodies. Data are presented as mean ± SEM, and $P < .05$ was considered significant.

Results

Very High Concentrations of IL6 Prevent IL1β- and IL6-Induced Reduction in Neurogenesis by Increasing IL8 Production

Cells were treated with low concentrations of cytokines to mimic the phenotype of healthy individuals (IL6 with IL1β or TNF-α, all 1 pg/mL) or high concentrations of cytokines to mimic the phenotype of depressed patients (IL6 5 pg/mL with IL1β 10 pg/mL or TNF-α 10 pg/mL); in addition, a very high concentration of IL6 (50 000 pg/mL) was used to mimic "anti-inflammatory" conditions (see Methods section for references explaining the chosen concentrations). As the results for IL1β and TNF-α are virtually identical, the Results section will focus on the combination of IL1β with IL6, whereas results for treatment with the combination of TNF-α with IL6 or for treatment with IL1β or TNF-α alone can be found in the supplementary Materials and in supplementary Figures 3 and 4.

Treatment of cells with high concentrations of both IL1β and IL6 (resembling depressed patients) decreased the percentage of DCX+ and Map2+ cells (respectively, –13%, $P < .05$ and –12%, $P < .05$; Figure 1a,b; vs treatment with low concentrations of both IL1β and IL6, resembling healthy individuals). However, treatment with high IL1β and a very high concentration of IL6 (50 000 pg/mL, resembling anti-inflammatory conditions) was able to prevent the decrease in DCX+ and Map2+ cells, respectively (+9%, $P < .05$ and +11%, $P < .05$; Figure 1a,b compared with high concentrations resembling depressed patients). Also, treatment with both high concentrations of IL1β and IL6 increased the percentage of CC3+ cells (+5%, $P < .05$; Figure 1c; vs low concentrations of IL1β and IL6), but in this case, treatment with high IL1β and a very high concentration of IL6 did not prevent this increase in CC3+cells (Figure 1c). This is consistent with our dose-response curve showing that the very high concentration of IL6 alone increased apoptosis (see supplementary Figure 1).

To investigate the mechanism underpinning the changes in neurogenesis described above, we characterize the effects of high concentrations of IL1β and IL6 (depressed patients) vs low concentrations (healthy individuals) on cytokine secretion in the supernatant of the cells and investigate whether any effects on cytokine secretion can be prevented by the very high concentration of IL6 (anti-inflammatory). We found that high concentrations of IL1β and IL6 upregulated their own production (Figure 2a,d), increased IL8 and IL13 (Figure 2e,h) and decreased IL2, IL12, IFN-γ, and TNF-α (Figure 2b,g,j,k) compared with low concentrations. Interestingly, treatment with high IL1β and the very high concentration of IL6 prevented the increase in IL8 (levels decreased from 39.8 pg/mL to 18.6 pg/mL, $P < .0001$; Figure 2e), but did not change the production of the other cytokines (Figure 2a,b,d,g,h,j,k).

To test whether the beneficial anti-inflammatory effect of the very high concentration of IL6 on neurogenesis indeed was due to the reduction of IL8 cytokine production, cells previously exposed to both high IL1β and IL6 (depressed patients) were co-treated with an antibody for IL8 (0.5 µg/mL). Indeed, much like the very high concentration of IL6, exposure to such antibody prevented the decrease in DCX+ and Map2+ cells (+14%, $P < .01$ and +20%, $P < .05$, vs high concentrations; supplementary Figure 5a,b).

Very High Concentrations of IL6 Prevent MIF- and IL6-Induced Reduction in Neurogenesis by Increasing IL1β Production

As in the experiments above, cells were treated with low concentrations of cytokines to mimic the phenotype of healthy individuals (IL6 1 pg/mL with MIF 10 pg/mL) or high concentrations of cytokines to mimic the phenotype of depressed patients (IL6 5 pg/mL with MIF 300 pg/mL); in addition, a very high concentration of IL6 (50 000 pg/mL) was used to mimic the "anti-inflammatory" phenotype. Results from treatment with MIF alone are described in the supplementary Materials and in supplementary Figure 6.

Treatment of cells with high concentrations of MIF and IL6 (depressed patients) did not affect the percentage of DCX+ cells but decreased the percentage of Map2+ cells (–6%, $P < .05$, Figure 3b, vs low MIF and IL6, healthy individuals). In contrast, treatment with high MIF and a very high concentration of IL6 prevented the decrease in Map2+ cells (+11%, $P < .05$, Figure 3b, compared with high concentrations resembling depressed patients). Also, treatment of cells with high concentrations of MIF and IL6 increased the percentage of CC3+ cells (+7%, $P < .05$, Figure 3c, vs low concentrations of MIF and IL6). However, and similar to experiments with IL1β, treatment with high MIF and a very high concentration of IL6 did not prevent the increase in CC3+cells caused by high concentrations (Figure 3c).

As previously, to investigate the mechanism underpinning the changes in neurogenesis described above, we characterized the effects of high concentrations of MIF and IL6 (depressed patients) vs low concentrations (healthy individuals)

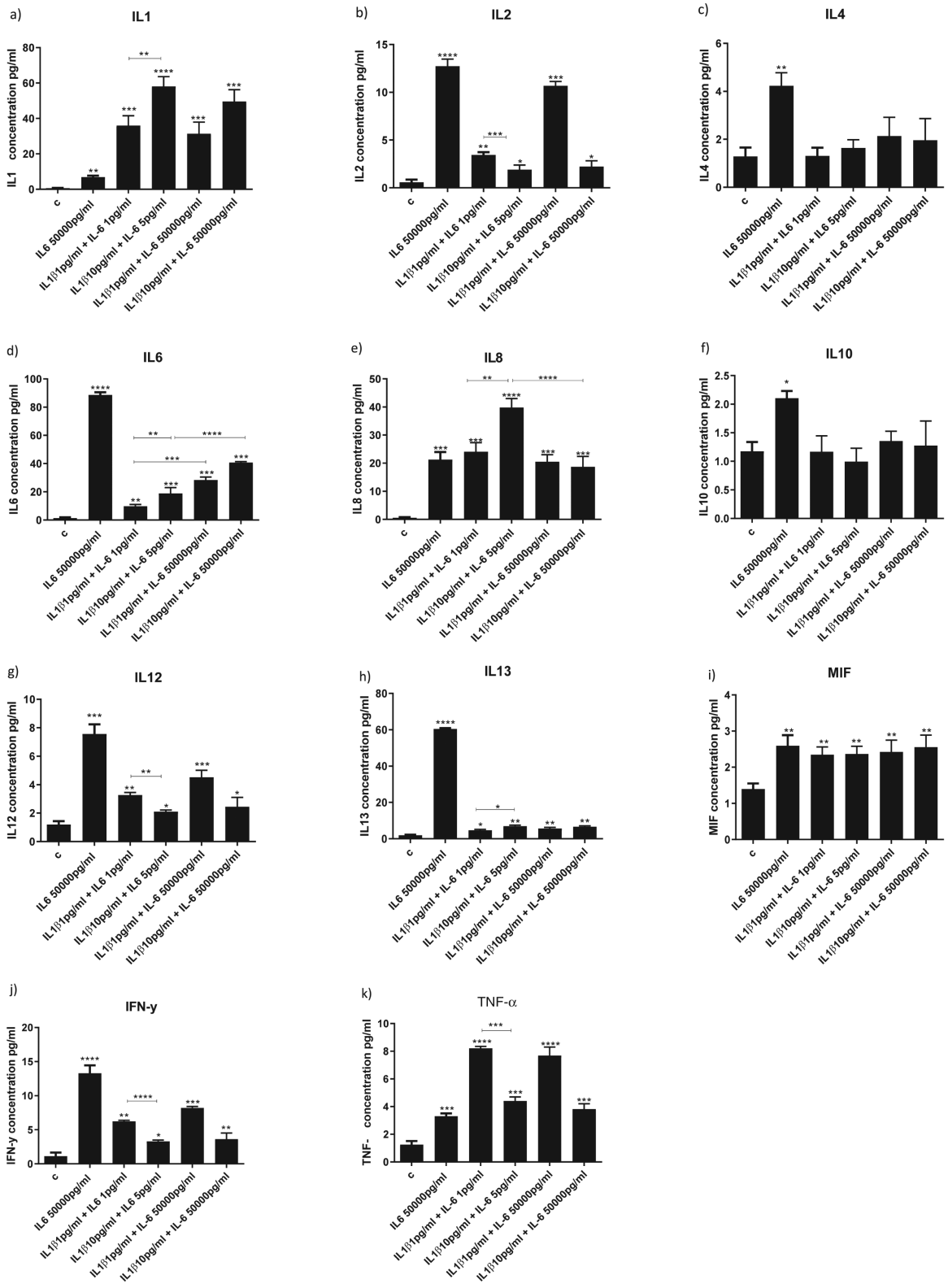

Figure 2. Production of cytokines in supernatant of cells exposed to low and high concentrations of interleukin 1beta (IL1β) with low, high, and very high concentrations of interleukin 6 (IL6). Concentrations of cytokines in supernatant of cells treated for 3 days during proliferation followed by 7 days during differentiation with low and high concentrations of IL1β (1 pg/mL, 10 pg/mL) and IL6 (1 pg/mL and 5 pg/mL) and very high concentration of IL6 (50000 pg/mL). Treatment alone with a very high concentration of IL6 increased levels of all cytokines (a–k). Treatment with high concentrations of both IL1β and IL6 significantly upregulated their own production (a, d), increased IL8 and IL13 (e, h), and decreased IL2, IL12, interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α) (b, g, j, k) compared with low concentrations of both IL1β and IL6. Treatment with high IL1β and a very high concentration of IL6 prevented the increase in IL8 caused by high IL1β and IL6 compared with low IL1β and IL6 (e) but did not change the production of the other cytokines (a, b, d, g, h, j, k). Two-way ANOVA with Bonferroni's post hoc test. Data are shown as mean ± SEM; $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, $^{****}P < .0001$, compared with vehicle treatment or as indicated.

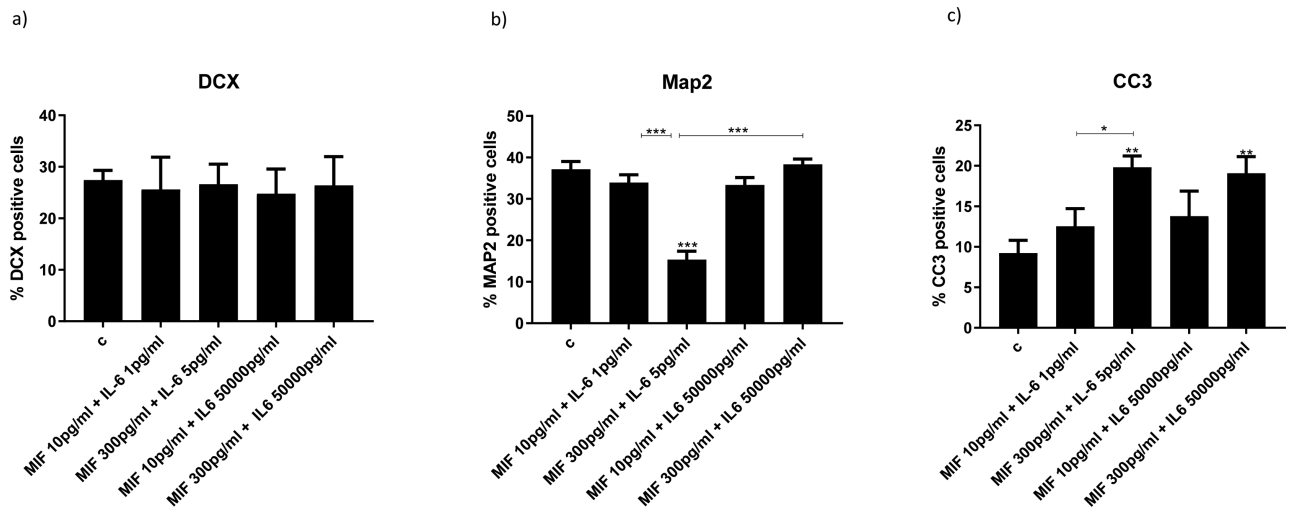

Figure 3. Very high concentrations of interleukin 6 (IL6) prevent Macrophage Migration Inhibitory Factor (MIF)- and IL6-induced reduction in neurogenesis. Treatment for 3 days during proliferation followed by 7 days during differentiation with high concentrations of MIF (10 and 300 pg/mL) and IL6 (1 and 5 pg/mL) did not affect the percentage of doublecortin (DCX)+cells but decreased the percentage of microtubule-associated protein 2 (Map2)+ cells compared with co-treatment with low concentrations of MIF and IL6 (a, b). In contrast, co-treatment with high MIF and a very high concentration of IL6 (50000 pg/mL) was able to prevent the decrease in Map2+ cells caused by treatment with high MIF and IL6 (b). Co-treatment of cells with high concentrations of MIF and IL6 increased the percentage of caspase3 (CC3)+ cells compared with co-treatment with low concentrations of MIF and IL6. However, co-treatment with MIF and a very high concentration of IL6 did not prevent the increase in CC3+cells caused by high MIF and IL6 (c). One-way ANOVA with Bonferroni's post hoc test. Data are shown as mean ± SEM; $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ compared with vehicle treatment or as indicated.

on cytokine secretion in the supernatant of the cells and investigated whether any effects on cytokine secretion can be prevented by the very high concentration of IL6 (anti-inflammatory). Similarly to the high IL1β and IL6, we found that high concentrations of MIF and IL6 upregulated their own production (Figure 4d,i), plus they significantly increased IL1β (Figure 4a) and decreased IL2, IL4, IL8, IL10, IL12, IL13, IFN-γ, and TNF-α (Figure 4b,c,e,f,g,h,j,k) compared with low concentrations of MIF and IL6. Interestingly, treatment with high MIF and a very high concentration of IL6 prevented the increase in the production of the pro-inflammatory IL1β (levels decreased from 12.8 pg/mL to 3.2 pg/mL, $P < .0001$, Figure 4a), but did not change the production of the other cytokines (Figure 4b–k).

Finally, to mimic whether the beneficial effect of a very high concentration of IL6 on neurogenesis was due to the reduction of IL1β cytokine production, cells previously exposed to high MIF and IL6 (depressed patients) were co-treated with an antibody for IL1β (0.1 μg/mL). Similar to treatment with the very high concentration of IL6, exposure to such antibody prevented a decrease in Map2+ cells (+15%, $P < .01$, vs high concentrations; supplementary Figure 7b).

Very High Concentrations of IL6 Are More Effective Than Antidepressants in an IL1β Plus MIF Co-Incubation Model of Treatment-Resistant Depression

Having just demonstrated the ability for a very high concentration of IL6 (50000 pg/mL) to exert neurogenic protective properties in the presence of high concentrations of either IL1β (10 pg/mL) or MIF (300 pg/mL), as found in depressed patients, we investigated whether exposing cells to both cytokines (IL1β and MIF), as described in clinical studies of treatment-resistant depressed patients (Cattaneo et al., 2013, 2016), would induce a different neurogenic phenotype in response to IL6 or antidepressants. Specifically, we compared the effect of the very high concentration of IL6, putatively anti-inflammatory and antidepressant in our cellular model, with the effect of 2 antidepressants, sertraline and venlafaxine (both 1 μM), which we previously showed to prevent the detrimental effects of IL1β in our in vitro cellular model (Borsini et al., 2017).

Treatment of cells with high concentrations of IL1β and MIF (resembling treatment-resistant depressed patients) reduced the percentage of DCX+ and Map2+ cells (respectively, –4%, $P < .01$, and –11%, $P < .001$, Figure 5a,b, vs treatment with low concentrations of IL1β and MIF resembling healthy individuals). As hypothesized, treatment with high IL1β and MIF and the very high concentration of IL6 prevented the decrease in DCX+ and Map2+ cells (+18% for both markers, $P < .001$, vs the high concentration resembling treatment-resistant patients; Figure 5a,b). Surprisingly, neither antidepressant treatment was able to prevent the decrease in DCX+ or Map2+cells induced by high IL1β and MIF (Figure 5b), even if alone they did stimulate neurogenesis (+6% in MAP2+ cells for both antidepressants, $P < .01$; Figure 5b), and we showed in previous studies that these antidepressants can either increase neurogenesis on their own (Anacker et al., 2011) or prevent the reduction in neurogenesis induced by IL1β alone (Borsini et al., 2017).

Also, treatment of cells with high concentrations of IL1β and MIF (treatment-resistant depressed patients) increased the percentage of CC3+ cells (+9%, $P < .001$; Figure 5c) compared with low IL1β and MIF (healthy individuals). Consistently with the effects with the cytokines alone (supplementary Figures 3 and 6), or indeed of IL6 alone (supplementary Figure 1), treatment with high IL1β and MIF and a very high concentration of IL6 did not prevent the increase in CC3+cells caused by high MIF and IL6 (Figure 5c). However, treatment with sertraline, but not venlafaxine, partially reduced the increase in CC3+ cells (–6%, $P < .05$, Figure 5c, compared with the high concentrations).

We then measured the effects of high concentrations of both IL1β and MIF (treatment-resistant depressed patients) vs low concentrations (healthy individuals) on cytokine secretion in supernatant of these cells. In particular, high concentrations

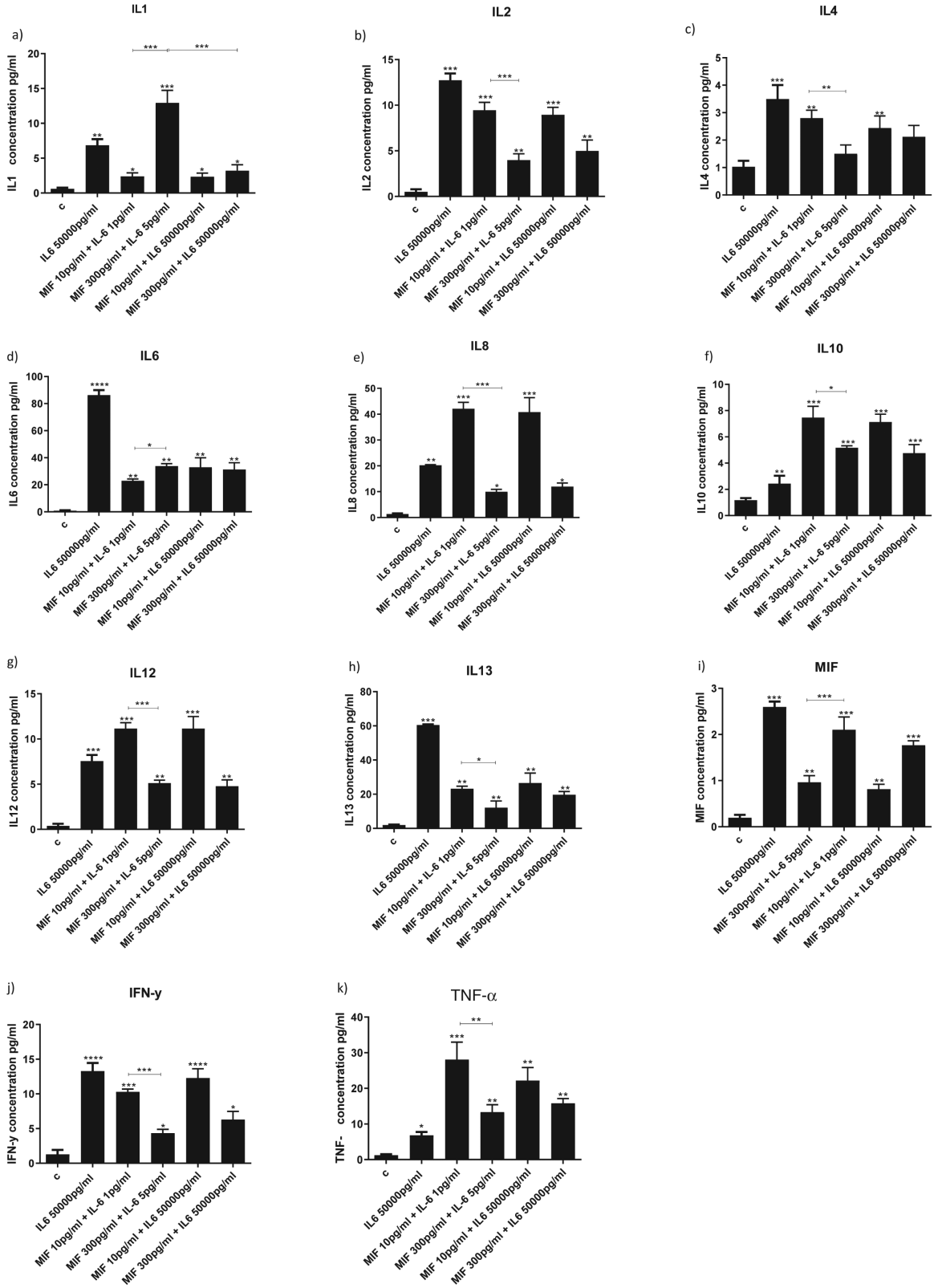

Figure 4. Production of cytokines in supernatant of cells exposed to low and high concentrations of Macrophage Migration Inhibitory Factor (MIF) with low, high, and very high concentrations of Interleukin 6 (IL6). Concentrations of cytokines in supernatant of cells treated for 3 days during proliferation followed by 7 days during differentiation with low and high concentrations of MIF (10 pg/mL, 300 pg/mL) and IL6 (1 pg/mL and 5 pg/mL), and very high concentration of IL6 (50 000 pg/mL). Treatment alone with a very high concentration of IL6 increased levels of all cytokines (a–k), whereas treatment with high concentrations of both MIF and IL6 significantly upregulated their own production (d, i), increased IL1β (a), and decreased IL2, IL4, IL8, IL10, IL12, IL13, interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α) (b, c, e, f, g, h, j, k) compared with low concentrations of MIF and IL6. Treatment with high MIF and a very high concentration of IL6 prevented the increase in IL1β caused by high MIF and IL6 compared with low MIF and IL6 (a), but did not change the production of the other cytokines (b–k). Two-way ANOVA with Bonferroni's post hoc test. Data are shown as mean ± SEM; $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, $^{****}P < .0001$, compared with vehicle treatment or as indicated.

of IL1β and MIF upregulated their own production (Figure 6a, i), and significantly decreased IL2, IL4, IL6, IL8, IL10, IL12, IL13, IFN-γ, and TNF-α (Figure 6b–k) compared with low concentrations. Interestingly, treatment with high IL1β and MIF and a very high concentration of IL6 increased the production of the anti-inflammatory IL4 compared with low IL1β and MIF (from 1.5 pg/mL to 3.4 pg/mL, $P < .0001$; Figure 6c). No changes were observed for the other cytokines (Figure 6a, b, d–k). These results were different from the effects of antidepressants, since sertraline together with high IL1β/MIF reduced the production of IL6 (from 13.02 pg/mL to 7.2 pg/mL, $P < .05$; Figure 6d) but did not change the production of the other cytokines, and venlafaxine did not have any effects at all (Figure 6a, b, c, e–k).

Finally, to test whether the beneficial effect on neurogenesis by the very high dose of IL6 in the presence of the IL1β and MIF together was due to the increase in IL4 and whether the beneficial effects on apoptosis by sertraline were due to a reduction in IL6, cells previously exposed to high IL1β and MIF were co-treated with either IL4 (3 pg/mL and 30 pg/mL) or an antibody for IL6 (0.1 μg/mL). Much like the very high dose of IL6, exposure to IL4 prevented the decrease in DCX+ and Map2+ cells (DCX: +6%, $P < .05$ for IL4 3 pg/mL, and +10%, $P < .05$ for IL4 30 pg/mL; Map2: +17%, $P < .01$ for IL4 3 pg/mL, and +24%, $P < .05$ for IL4 30 pg/mL) (supplementary Figure 8a,b). Similarly, much like sertraline, treatment with IL6A partially prevented the increase in CC3+ cells (+4%, $P < .01$, supplementary Figure 8f, compared with high IL1β and MIF).

Discussion

In this study, we provide the first evidence, to our knowledge, that treatment with high concentrations of IL6 and IL1β or MIF, resembling levels found in blood and CSF of depressed patients, decreases neurogenesis when compared with low concentrations of the same cytokines and resembling healthy subjects. Interestingly, this effect is mediated via different mechanisms based on the cytokine/s involved, with increased production of the pro-inflammatory cytokines IL8 and IL1β on treatment with, respectively, IL1β and IL6 together or MIF and IL6 together, and reduced production of the anti-inflammatory cytokine IL4 on treatment with IL1β and MIF together. Interestingly, treatment with a very high concentration of IL6, resembling that in the blood of patients during putatively anti-inflammatory/antidepressant conditions like physical exercise and hyperthermia (Raison et al., 2018), is able to prevent the decrease in neurogenesis in all 3 of these experimental conditions, that is, with IL1β, MIF, and IL1β and MIF together. Moreover, this very high concentration of IL6 does so by reversing the specific mechanisms activated by the 3 experimental conditions, that is, by reducing IL8 with IL1β, reducing IL1β with MIF, and increasing IL4 with IL1β and MIF. Finally, using IL1β and MIF in co-treatment (which we propose as a model of treatment-resistant depression) we also show that reduction in neurogenesis induced by this model can respond to the very high dose of IL6 but not to the antidepressants sertraline and venlafaxine.

This study identifies distinct and unique anti-inflammatory and antidepressant properties of IL6, which follow a U-shaped curve of its concentrations and varies in combinations with other inflammatory cytokines. When cells are treated with different concentrations of IL6 alone, intermediate concentrations decrease neurogenesis (50, 500, and 5000 pg/mL), but 1 pg/mL, 5 pg/mL, and the very high 50000-pg/mL concentrations do not cause any changes. This is consistent with other studies showing that IL6 can either not change or enhance neuronal differentiation and that these findings are dependent on its concentration (Johansson et al., 2008; Islam et al., 2009; Zonis et al.,

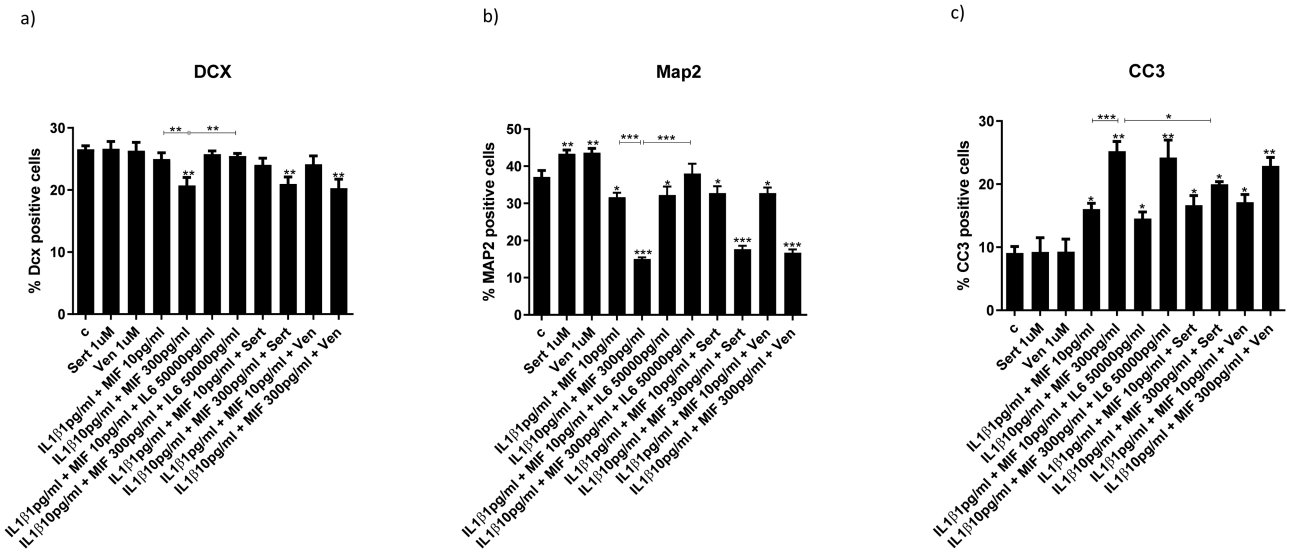

Figure 5. Very high concentrations of interleukin 6 (IL6) are more effective than antidepressants in an interleukin 1beta (IL1β) plus Macrophage Migration Inhibitory Factor (MIF) co-incubation model of treatment-resistant depression. Treatment for 3 days during proliferation followed by 7 days during differentiation with high concentrations of IL1β (10 pg/mL) and MIF (300 pg/mL) significantly reduced the percentage of doublecortin (DCX)+ and microtubule-associated protein 2 (Map2)+ cells compared with co-treatment with low concentrations of IL1β (1 pg/mL) and MIF (10 pg/mL). In contrast, co-treatment with high IL1β and MIF and a very high concentration of IL6 was able to prevent the decrease in DCX+ and Map2+ cells caused by treatment with high IL1β and MIF. Although, treatment alone with sertraline (Sert) and venlafaxine (Ven) (1 μM both) increased the percentage of Map2+cells compared with control, co-treatment of high IL1β and MIF with both antidepressants did not cause any changes in either DCX+ or Map2+cells compared with high IL1β and MIF (a, b). Co-treatment of cells with high concentrations of IL1β and MIF increased the percentage of caspase 3 (CC3)+ cells compared with low concentrations of IL1β and MIF. However, co-treatment with high IL1β and MIF and a very high concentration of IL6 did not prevent the increase in CC3+cells caused by high MIF and IL6. Instead, co-treatment with high IL1β and MIF and venlafaxine, but not sertraline, partially reduced the increase in CC3+cells caused by high IL1β and MIF (c). Two-way ANOVA with Bonferroni's post hoc test. Data are shown as mean ± SEM; *$P < .05$, **$P < .01$, ***$P < .001$, compared with vehicle treatment or as indicated.

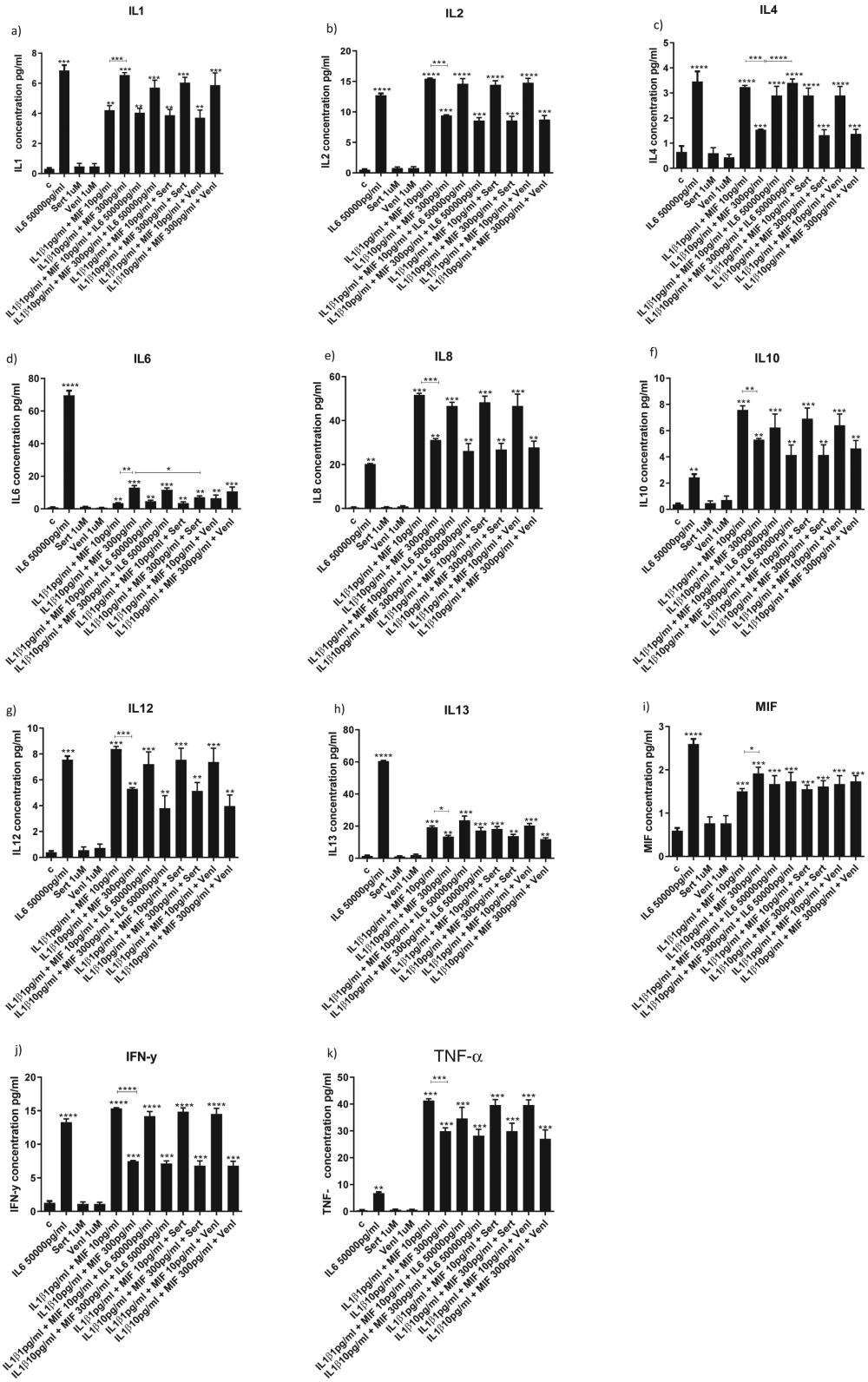

Figure 6. Production of cytokines in supernatant of cells exposed to low and high concentrations of IL1β and Macrophage Migration Inhibitory Factor (MIF) with very high concentrations of Interleukin 6 (IL6) or antidepressants. Concentrations of cytokines in supernatant of cells treated for 3 days during proliferation followed by 7 days during differentiation with low and high concentrations of IL1β (1 pg/mL, 10 pg/mL) and MIF (10 pg/mL and 300 pg/mL) and very high concentration of IL6 (50 000 pg/mL) or sertraline (Sert) and venlafaxine (Ven) (1 μM, both). Treatment with a very high concentration of IL6 increased the levels of all cytokines (a–k); however, sertraline and venlafaxine did not change levels of any cytokine (a–k). Treatment with high concentrations of IL1β and MIF significantly upregulated their own production (a, i) and decreased IL2, IL4, IL6, IL8, IL10, IL12, IL13, interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α) (b–h, j, k) compared with low concentrations of IL1β and MIF. Interestingly, treatment with high IL1β and MIF and a very high concentration of IL6 decreased the production of the anti-inflammatory IL4 caused by high IL1β and MIF compared with low IL1β and MIF (c). No changes were observed on the other cytokines (a, b, d–k). However, treatment with high IL1β and MIF and sertraline, but not venlafaxine, reduced the production of IL6 caused by high IL1β and MIF compared with treatment with low IL1β and MIF (d) but did not change the production of the other cytokines (a–c, e –k). Two-way ANOVA with Bonferroni's post hoc test. Data are shown as mean ± SEM; $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, $^{****}P < .0001$, compared with vehicle treatment or as indicated.

2013; Borsini et al., 2015). Similarly, when cells are exposed to low concentrations of IL6 (1 pg/mL) together with low concentrations of IL1β (1 pg/mL) or MIF (10 pg/mL), as found in healthy individuals (Lindqvist et al., 2009; Pawlitzki et al., 2018), we do not observe any changes in neurogenesis. In contrast, high concentrations of IL6 (5 pg/mL) with either IL1β (10 pg/mL) or MIF (300 pg/mL), as found in depressed patients (Piletz et al., 2009; Hestad et al., 2016; Kranaster et al., 2018; Tsuboi et al., 2018), decrease dramatically the percentage of newly generated neurons compared not only with low concentrations of the same combination of cytokines but also with the cytokines alone. Indeed, high concentrations of IL6 (5 pg/mL) alone do not affect neurogenesis but added to either IL1β (10 pg/mL) or MIF (300 pg/mL) further reduces neurogenesis beyond the effects of the 2 cytokines alone (from –12% to –20% for IL1β and from –18% to –22% for MIF). This confirms the notion that IL6 may shift towards a more pro-inflammatory status when in the presence of other pro-inflammatory cytokines, like IL1β or MIF, but not when used alone. To our surprise, however, when we expose cells to IL1β or MIF and a very high concentration of IL6 (50 000 pg/mL, a concentration that alone does not affect neurogenesis), the IL1β- or MIF-induced reduction in neurogenesis is fully prevented, and levels return similar to those elicited by low concentrations of IL6 with IL1β or MIF (healthy individuals).

One of the possible mechanisms through which very high levels of IL6 exert neuroprotective properties could be via regulation of downstream molecules involved in the inflammatory response. When inflammation is triggered, high concentrations of IL6 are released into circulation together with other cytokines, including IL1β and TNF-α (Raison et al., 2018). This process, known as "trans-signaling," accounts for the inflammatory actions of IL6, which include activation of neutrophils, as well as production of C-reactive protein and other acute phase proteins (Del Giudice and Gangestad, 2018). On the other hand, during putatively anti-inflammatory/antidepressant conditions, like exercise, fasting, or hyperthermia, very high concentrations of IL6 are produced (up to 1000-fold higher than baseline), and these can inhibit the release of the same pro-inflammatory cytokines, IL1β and TNF-α, and induce the production of IL10, the body's primary anti-inflammatory cytokine (Del Giudice and Gangestad, 2018; Raison et al., 2018). This process, also called "classical signaling pathway," is the main anti-inflammatory mode of action of IL6. For example, in healthy individuals exposed to acute exercise, IL6 is stimulated in very high amounts, at least 100-fold higher than baseline (Pedersen and Febbraio, 2008), while IL1β and TNF-α levels are reduced and the anti-inflammatory IL10 and IL1 receptor antagonist are increased (Raison et al., 2018). Similarly, in healthy individuals, mice exposed to fasting, or depressed patients receiving hyperthermia, IL6 levels are also very high (up to 1000-fold than baseline), whereas IL1β and TNF-α concentrations are relatively low (Raison et al., 2018). Therefore, a similar pattern in IL6 production can be seen across all these interventions (physical exercise, hyperthermia, and fasting), which are known to have both antidepressant and mood-elevating properties. In fact, ketamine, which is able to produce a rapid and profound antidepressant effect, also acutely increases circulating levels of IL6 (Park et al., 2016). Interestingly, IL6 can have beneficial properties not only in the context of depression but also in other pathological conditions such as neurodegenerative disorders. For example, in an in vitro rat model of Parkinson's disease, an increase in IL6 concentration (up to 100-fold higher than baseline) protects against neurotoxic effects induced by 1-methyl-4-phenylpyridinium, a compound that mimics the selective neuronal loss observed in Parkinson's disease (Hama et al., 1991). Similarly, in an in vivo transgenic mouse model of Alzheimer's disease, overproduction of IL6 (up to 10-fold higher than baseline) attenuates beta-amyloid peptide deposition and enhances plaque clearance (Chakrabarty et al., 2010). Overall, this confirms the notion, eloquently proposed by Raison et al, 2018 that IL6, although being regarded as a "bad kid" for its pro-inflammatory properties, is in fact a "good kid" able to exert anti-inflammatory and anti-depressant properties when expressed at very high concentrations, through which it reduces (or maintains) the concentration of other concomitant pro-inflammatory cytokines to relatively low and harmless levels (Raison et al., 2018).

This previous evidence is also in line with our mechanistic in vitro experiments. Treatment with a very high concentration of IL6 reduces the production of the pro-inflammatory cytokines IL8 and IL1β (induced by, respectively, high IL1β or MIF) and increases the production of IL4 (which is reduced by co-treatment with high IL1β and MIF). Clinical studies show that the concentrations of both IL8 and IL1β in peripheral blood and CSF are higher in depressed patients compared with healthy individuals, whereas it is the opposite for IL4, where levels are lower in depressed than in healthy individuals (Piletz et al., 2009; Cattaneo et al., 2013; Hestad et al., 2016; Tsuboi et al., 2018). Interestingly, in our study, the concentrations of IL8, IL1β, and IL4 in the cell supernatant of the experimental conditions resembling depressed patients were very similar to the actual concentrations found in blood and CSF of depressed patients (Piletz et al., 2009; Hestad et al., 2016; Tsuboi et al., 2018), confirming the validity of our model as "depression in a dish." Previous studies of in vitro and in vivo models of depression have also found that IL8 and IL1β can detrimentally reduce neurogenesis and that IL4 can instead increase both neuronal and glial differentiation (Bluthe et al., 2002; Goshen et al., 2008; Koo and Duman, 2008; Borsini et al., 2015, 2017; Ryu et al., 2015; Wang et al., 2018). Moreover, pharmacological inhibition of IL8 receptor CXC chemokine receptor 2, or inhibition of IL1β signal via treatment with IL1RA, can prevent the decrease in neurogenesis and reduce depressive-like behaviors caused by IL8 and IL1β (Wang et al., 2007; Goshen et al., 2008; Koo and Duman, 2008; Ryu et al., 2015), therefore confirming the involvement of these cytokines not only in the pathogenesis but also in the treatment of depressive disorders.

Although both IL8 and IL4 are of particular interest in the context of depression, there is still a lack of understanding of their exact role in this disorder. One study has examined the expression of IL8 in response to ex vivo lipopolysaccharide stimulation of whole blood from depressed patients and found that a high IL8 level is strongly associated with disorder status, even after adjustment for several patients' lifestyle and health factors (Vogelzangs et al., 2016). It is still unknown, however, which signaling pathways may be involved in these effects. IL8 induces activation of several immune-related transcription factors, including nuclear factor kappa-light-chain-enhancer of activated B cells and signal transducer and activator of transcription 1 (Manna and Ramesh, 2005; Guo et al., 2017), which we have previously shown to be involved in the detrimental effects exerted by cytokines on neurogenesis in our in vitro model of "depression in a dish" (Horowitz et al., 2014; Borsini et al., 2018). In contrast to IL8, IL4 has beneficial properties, with high levels of the cytokine known to be associated with reduced depressive and anxiety symptoms in patients (Cattaneo et al., 2013; Hou et al., 2017). IL4 is a very well-known anti-inflammatory cytokine that is able to shift the production of T helper 1 (Th1)

type pro-inflammatory cytokines, like IL2 and IL12, to Th2 type anti-inflammatory cytokines, such as IL10 (Sutcigil et al., 2007), while a high Th1:Th2 activation ratio is often observed in patients with major depression (Myint et al., 2005). In our study, treatment with high IL1β and MIF and the very high concentration of IL6 increased both neurogenesis and concentration of IL4 to levels originally found with low IL1β and MIF (resembling healthy individuals); however, concentrations of IL2, IL12, and IL10 remained unchanged. This perhaps suggests that, in the presence of very high concentrations of IL6, the way IL4 exerts its neurogenic properties may not be mediated by production or inhibition of those candidate cytokines but instead by other mechanisms. IL4 can in fact increase the expression of several neurogenic factors, including growth factor receptors like EGF receptor and fibroblast growth factor receptor 1 (Puri et al., 2005), which can positively affect neurogenesis (Oliveira et al., 2013). Overall, this evidence highlights the importance of both IL8 and IL4 in the context of depression, with their pro- and anti-inflammatory properties, respectively, reflected in their potential ability to contribute to (IL8) or protect from (IL4) the development of depression.

On their own, the cytokines IL1β and MIF also deserve attention for their role as predictors of antidepressant treatment response. In particular, findings from our previous publications have shown that patients with high baseline mRNA levels of both IL1β or MIF, but not other cytokines, are less likely to respond to antidepressant treatment (Cattaneo et al., 2013, 2016), making treatment with both IL1β and MIF a good in vitro model of treatment-resistant depression. In our study, high concentrations of both IL1β and MIF decrease neurogenesis and increase apoptosis, not only compared with low concentrations of IL1β and MIF but also compared with either cytokines alone. Indeed, high concentrations of IL1β or MIF alone reduce neurogenesis (–12% and –18%, respectively, vs control condition) and increase apoptosis (+8% and +11%, respectively, vs control condition), and this effect becomes even stronger when both IL1β and MIF are used in co-treatment (–22% neurogenesis and +16% apoptosis vs control condition). Moreover, these effects are mediated by a reduction in IL4 (for neurogenesis) and an increase in IL6 (for apoptosis). This is in accordance with previous evidence suggesting that IL1β and MIF can indeed govern the production of several downstream cytokines, including IL4 and IL6, as well as other molecules, such as Endothelial Growth Factor and Notch, which are involved in the regulation of cell proliferation, neurogenesis, and neuroplasticity (Leemans et al., 2011; Anacker et al., 2013a; Radi et al., 2014).

Since high levels of IL1β and MIF predict a lower response rate to antidepressants in patients, we investigated the effect of treatment with 2 antidepressants, sertraline and venlafaxine, in our in vitro model and compared that with treatment with the very high concentration of IL6. Interestingly, neither antidepressant was able to prevent changes in neurogenesis caused by the high concentrations of IL1β and MIF, while treatment with a very high concentration of IL6 did prevent the reduction in neurogenesis and increased IL4 production. This is a major finding, as it shows for the first time to our knowledge, albeit in an experimental model, that a very high concentration of IL6 can be more effective than SSRI and SNRI antidepressants in preventing inflammation-induced reduction in hippocampal neurogenesis, a mechanism involved both in depression and antidepressant treatment response (Santarelli et al., 2003; Boldrini et al., 2009, 2014). Therefore, this suggests that treatment with very high concentrations of IL6 might be a suitable therapeutic approach in treatment-resistant depression, especially for those patients having high baseline levels of inflammation—consistent with the aforementioned clinical evidence that similarly very high concentrations of IL6 are induced by putative antidepressant interventions like hyperthermia and fasting (Wueest et al., 2014; Raison et al., 2018).

Of course, we acknowledge the limitation that this is an in vitro system with an immortalized cell line. However, while theoretically this system may differ from the scenario of an adult in vivo environment and the adult neurogenic niche, over the years we have been able to replicate all our results with this in vitro model in either animal or clinical studies, including changes in neurogenesis by cortisol, IL1β, IFN-α, and antidepressants and changes in stress- and antidepressant-regulated genes in both the whole-blood mRNA of depressed patients and the hippocampal mRNA of animal models of depression (Anacker et al., 2011, 2013a, 2013b; Zunszain et al., 2012; Horowitz et al., 2014; Borsini et al., 2017). Therefore, we are confident that our results are relevant to the human brain. Of note, the majority of our progenitor cells can differentiate into neurons (70%–80%), where cytokines can be constitutively expressed (Breder et al., 1994; Gadient and Otten, 1994; Ringheim et al., 1995; Galic et al., 2012). Our cells also differentiate into astrocytes (20%–30%), but not in microglia. In this study, we did not assess the effect of the cytokines on astrogliogenesis, as we decided to primarily focus on neuronal differentiation and cell apoptosis. Considering our previous study showing that cortisol reduces astrogliogenesis (Anacker et al., 2013a), in our future studies we aim to extend these findings and explore which cell type (neuron and/or astrocyte) is most responsible for subsequent downstream cytokine production as well as glia-related changes on exposure to different concentrations and treatment combinations of the above cytokines.

In summary, our study reveals the ability of very high concentrations of IL6 to prevent reduction in neurogenesis caused by high concentrations of IL6 with IL1β or MIF (resembling depressed patients) or by high concentrations of both IL1β and MIF together (resembling treatment resistant depressed patients) via regulation of distinct signaling molecules. Overall, our results demonstrate the ability for IL6 to exert both pro- and anti-inflammatory as well as (potentially) antidepressant properties, which are dependent on its concentration and the various combinations with other inflammatory cytokines.

Supplementary Materials

Supplementary data are available at *International Journal of Neuropsychopharmacology (IJNPPY)* online.

Acknowledgments

Dr Alessandra Borsini and Professor Carmine M. Pariante are funded by the UK Medical Research Council (grants MR/L014815/1, MR/J002739/1, and MR/N029488/1), the European Commission Horizon 2020 (grant SC1-BHC-01-2019), and the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London. Professor Pariante is a NIHR Senior Investigator (2017–2025).

Statement of Interest

Dr Alessandra Borsini and Professor Carmine M. Pariante have received research funding from Johnson and Johnson for

research on depression and inflammation, which included cellular work (2012–2018); moreover, Professor Pariante is funded by a Wellcome Trust strategy award to the Neuroimmunology of Mood Disorders and Alzheimer's Disease (NIMA) Consortium (104025), which is also funded by Janssen, GlaxoSmithKline, Lundbeck, and Pfizer. The work presented in this paper is unrelated to this funding.

References

Anacker C, Zunszain PA, Cattaneo A, Carvalho LA, Garabedian MJ, Thuret S, Price J, Pariante CM (2011) Antidepressants increase human hippocampal neurogenesis by activating the glucocorticoid receptor. Mol Psychiatry 16:738–750.

Anacker C, Cattaneo A, Luoni A, Musaelyan K, Zunszain PA, Milanesi E, Rybka J, Berry A, Cirulli F, Thuret S, Price J, Riva MA, Gennarelli M, Pariante CM (2013a) Glucocorticoid-related molecular signaling pathways regulating hippocampal neurogenesis. Neuropsychopharmacology 38:872–883.

Anacker C, Cattaneo A, Musaelyan K, Zunszain PA, Horowitz M, Molteni R, Luoni A, Calabrese F, Tansey K, Gennarelli M, Thuret S, Price J, Uher R, Riva MA, Pariante CM (2013b) Role for the kinase SGK1 in stress, depression, and glucocorticoid effects on hippocampal neurogenesis. Proc Natl Acad Sci U S A 110:8708–8713.

Bluthé RM, Lestage J, Rees G, Bristow A, Dantzer R (2002) Dual effect of central injection of recombinant rat interleukin-4 on lipopolysaccharide-induced sickness behavior in rats. Neuropsychopharmacology 26:86–93.

Boldrini M, Underwood MD, Hen R, Rosoklija GB, Dwork AJ, John Mann J, Arango V (2009) Antidepressants increase neural progenitor cells in the human hippocampus. Neuropsychopharmacology 34:2376–2389.

Boldrini M, Butt TH, Santiago AN, Tamir H, Dwork AJ, Rosoklija GB, Arango V, Hen R, Mann JJ (2014) Benzodiazepines and the potential trophic effect of antidepressants on dentate gyrus cells in mood disorders. Int J Neuropsychopharmacol 17:1923–1933.

Borsini A, Zunszain PA, Thuret S, Pariante CM (2015) The role of inflammatory cytokines as key modulators of neurogenesis. Trends Neurosci 38:145–157.

Borsini A, Alboni S, Horowitz MA, Tojo LM, Cannazza G, Su KP, Pariante CM, Zunszain PA (2017) Rescue of IL-1β-induced reduction of human neurogenesis by omega-3 fatty acids and antidepressants. Brain Behav Immun 65:230–238.

Borsini A, Cattaneo A, Malpighi C, Thuret S, Harrison NA, Zunszain PA, Pariante CM; MRC ImmunoPsychiatry Consortium (2018) Interferon-Alpha reduces human hippocampal neurogenesis and increases apoptosis via activation of distinct STAT1-dependent mechanisms. Int J Neuropsychopharmacol 21:187–200.

Borsini A, Pariante CM, Zunszain PA, Hepgul N, Russell A, Zajkowska Z, Mondelli V, Thuret S (2019) The role of circulatory systemic environment in predicting interferon-alpha-induced depression: the neurogenic process as a potential mechanism. Brain Behav Immun 81:220–227.

Breder CD, Hazuka C, Ghayur T, Klug C, Huginin M, Yasuda K, Teng M, Saper CB (1994) Regional induction of tumor necrosis factor alpha expression in the mouse brain after systemic lipopolysaccharide administration. Proc Natl Acad Sci U S A 91:11393–11397.

Capuron L, Neurauter G, Musselman DL, Lawson DH, Nemeroff CB, Fuchs D, Miller AH (2003) Interferon-alpha-induced changes in tryptophan metabolism. relationship to depression and paroxetine treatment. Biol Psychiatry 54:906–914.

Capuron L, Pagnoni G, Demetrashvili MF, Lawson DH, Fornwalt FB, Woolwine B, Berns GS, Nemeroff CB, Miller AH (2007) Basal ganglia hypermetabolism and symptoms of fatigue during interferon-alpha therapy. Neuropsychopharmacology 32:2384–2392.

Cattaneo A, Gennarelli M, Uher R, Breen G, Farmer A, Aitchison KJ, Craig IW, Anacker C, Zunsztain PA, McGuffin P, Pariante CM (2013) Candidate genes expression profile associated with antidepressants response in the GENDEP study: differentiating between baseline "predictors" and longitudinal "targets." Neuropsychopharmacol 38:377–385.

Cattaneo A, Ferrari C, Uher R, Bocchio-Chiavetto L, Riva MA, Consortium MRCI, Pariante CM (2016) Absolute measurements of macrophage migration inhibitory factor and interleukin-1-beta mRNA levels accurately predict treatment response in depressed patients. Int J Neuropsychopharmacol 19:pyw045.

Chakrabarty P, Jansen-West K, Beccard A, Ceballos-Diaz C, Levites Y, Verbeeck C, Zubair AC, Dickson D, Golde TE, Das P (2010) Massive gliosis induced by interleukin-6 suppresses Abeta deposition in vivo: evidence against inflammation as a driving force for amyloid deposition. Faseb J 24:548–559.

Del Giudice M, Gangestad SW (2018) Rethinking IL-6 and CRP: why they are more than inflammatory biomarkers, and why it matters. Brain Behav Immun 70:61–75.

Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, Lanctôt KL (2010) A meta-analysis of cytokines in major depression. Biol Psychiatry 67:446–457.

Felger JC, Lotrich FE (2013) Inflammatory cytokines in depression: neurobiological mechanisms and therapeutic implications. Neuroscience 246:199–229.

Gadient RA, Otten U (1994) Identification of interleukin-6 (IL-6)-expressing neurons in the cerebellum and hippocampus of normal adult rats. Neurosci Lett 182:243–246.

Galic MA, Riazi K, Pittman QJ (2012) Cytokines and brain excitability. Front Neuroendocrinol 33:116–125.

Goshen I, Kreisel T, Ben-Menachem-Zidon O, Licht T, Weidenfeld J, Ben-Hur T, Yirmiya R (2008) Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression. Mol Psychiatry 13:717–728.

Guo Y, Zang Y, Lv L, Cai F, Qian T, Zhang G, Feng Q (2017) IL-8 promotes proliferation and inhibition of apoptosis via STAT3/AKT/NF-κB pathway in prostate cancer. Mol Med Rep 16:9035–9042.

Hama T, Kushima Y, Miyamoto M, Kubota M, Takei N, Hatanaka H (1991) Interleukin-6 improves the survival of mesencephalic catecholaminergic and septal cholinergic neurons from postnatal, two-week-old rats in cultures. Neuroscience 40:445–452.

Hepgul N, Cattaneo A, Agarwal K, Baraldi S, Borsini A, Bufalino C, Forton DM, Mondelli V, Nikkheslat N, Lopizzo N, Riva MA, Russell A, Hotopf M, Pariante CM (2016) Transcriptomics in interferon-alpha-treated patients identifies inflammation-, neuroplasticity- and oxidative stress-related signatures as predictors and correlates of depression. Neuropsychopharmacol 41:2502–2511.

Hepgul N, Pariante CM, Baraldi S, Borsini A, Bufalino C, Russell A, Agarwal K, Cleare AJ, Forton DM, Henderson M, Mondelli V, Ranjith G, Hotopf M (2018) Depression and anxiety in patients receiving interferon-alpha: the role of illness perceptions. J Health Psychol 23:1405–1414.

Hestad KA, Engedal K, Whist JE, Aukrust P, Farup PG, Mollnes TE, Ueland T (2016) Patients with depression display cytokine levels in serum and cerebrospinal fluid similar to patients

with diffuse neurological symptoms without a defined diagnosis. Neuropsychiatr Dis Treat 12:817–822.

Horowitz MA, Wertz J, Zhu D, Cattaneo A, Musaelyan K, Nikkheslat N, Thuret S, Pariante CM, Zunszain PA (2014) Antidepressant compounds can be both pro- and anti-inflammatory in human hippocampal cells. Int J Neuropsychopharmacol 18:pyu076.

Hou R, Garner M, Holmes C, Osmond C, Teeling J, Lau L, Baldwin DS (2017) Peripheral inflammatory cytokines and immune balance in generalised anxiety disorder: case-controlled study. Brain Behav Immun 62:212–218.

Islam O, Gong X, Rose-John S, Heese K (2009) Interleukin-6 and neural stem cells: more than gliogenesis. Mol Biol Cell 20:188–199.

Johansson S, Price J, Modo M (2008) Effect of inflammatory cytokines on major histocompatibility complex expression and differentiation of human neural stem/progenitor cells. Stem Cells 26:2444–2454.

Koo JW, Duman RS (2008) IL-1beta is an essential mediator of the antineurogenic and anhedonic effects of stress. Proc Natl Acad Sci U S A 105:751–756.

Kranaster L, Hoyer C, Aksay SS, Bumb JM, Müller N, Zill P, Schwarz MJ, Sartorius A (2018) Antidepressant efficacy of electroconvulsive therapy is associated with a reduction of the innate cellular immune activity in the cerebrospinal fluid in patients with depression. World J Biol Psychiatry 19:379–389.

Leemans JC, Cassel SL, Sutterwala FS (2011) Sensing damage by the NLRP3 inflammasome. Immunol Rev 243:152–162.

Lindqvist D, Janelidze S, Hagell P, Erhardt S, Samuelsson M, Minthon L, Hansson O, Björkqvist M, Träskman-Bendz L, Brundin L (2009) Interleukin-6 is elevated in the cerebrospinal fluid of suicide attempters and related to symptom severity. Biol Psychiatry 66:287–292.

Manna SK, Ramesh GT (2005) Interleukin-8 induces nuclear transcription factor-kappaB through a TRAF6-dependent pathway. J Biol Chem 280:7010–7021.

Miller AH, Raison CL (2016) The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nat Rev Immunol 16:22–34.

Myint AM, Leonard BE, Steinbusch HW, Kim YK (2005) Th1, Th2, and Th3 cytokine alterations in major depression. J Affect Disord 88:167–173.

Oliveira SL, Pillat MM, Cheffer A, Lameu C, Schwindt TT, Ulrich H (2013) Functions of neurotrophins and growth factors in neurogenesis and brain repair. Cytometry A 83:76–89.

Osimo EF, Pillinger T, Rodriguez IM, Khandaker GM, Pariante CM, Howes OD (2020) Inflammatory markers in depression: a meta-analysis of mean differences and variability in 5166 patients and 5083 controls. Brain Behav Immun 87:901–909.

Park M, Luckenbaugh DA, Newman LE, Niciu MJ, Lefler MS, Machado-Vieira R, Zarate CA (2016) Change in cytokine levels is not associated with antidepressant response to ketamine in patients with treatment resistant depression. Biol Psych 79:69s–70s.

Pawlitzki M, Sweeney-Reed CM, Meuth SG, Reinhold D, Neumann J (2018) CSF macrophage migration inhibitory factor levels did not predict steroid treatment response after optic neuritis in patients with multiple sclerosis. Plos One 13:e0207726.

Pedersen BK, Febbraio MA (2008) Muscle as an endocrine organ: focus on muscle-derived interleukin-6. Physiol Rev 88:1379–1406.

Piletz JE, Halaris A, Iqbal O, Hoppensteadt D, Fareed J, Zhu H, Sinacore J, Devane CL (2009) Pro-inflammatory biomakers in depression: treatment with venlafaxine. World J Biol Psychiatry 10:313–323.

Puri S, Joshi BH, Sarkar C, Mahapatra AK, Hussain E, Sinha S (2005) Expression and structure of interleukin 4 receptors in primary meningeal tumors. Cancer 103:2132–2142.

Radi E, Formichi P, Battisti C, Federico A (2014) Apoptosis and oxidative stress in neurodegenerative diseases. J Alzheimers Dis 42 Suppl 3:S125–S152.

Raison C (2017) Inflammation in treatment resistant depression: challenges and opportunities. Biol Psych 81:S171–S171.

Raison CL, Knight JM, Pariante C (2018) Interleukin (IL)-6: a good kid hanging out with bad friends (and why sauna is good for health). Brain Behav Immun 73:1–2.

Raison CL, Miller AH (2013) Role of inflammation in depression: implications for phenomenology, pathophysiology and treatment. Mod Trends Pharmacopsychiatry 28:33–48.

Ringheim GE, Burgher KL, Heroux JA (1995) Interleukin-6 mRNA expression by cortical neurons in culture: evidence for neuronal sources of interleukin-6 production in the brain. J Neuroimmunol 63:113–123.

Ryu JK, Cho T, Choi HB, Jantaratnotai N, McLarnon JG (2015) Pharmacological antagonism of interleukin-8 receptor CXCR2 inhibits inflammatory reactivity and is neuroprotective in an animal model of Alzheimer's disease. J Neuroinflammation 12:144.

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301:805–809.

Sutcigil L, Oktenli C, Musabak U, Bozkurt A, Cansever A, Uzun O, Sanisoglu SY, Yesilova Z, Ozmenler N, Ozsahin A, Sengul A (2007) Pro- and anti-inflammatory cytokine balance in major depression: effect of sertraline therapy. Clin Dev Immunol 2007:76396.

Tsuboi H, Sakakibara H, Minamida Y, Tsujiguchi H, Matsunaga M, Hara A, Nakamura H (2018) Elevated levels of serum IL-17A in community-dwelling women with higher depressive symptoms. Behav Sciences 8:102.

Vogelzangs N, de Jonge P, Smit JH, Bahn S, Penninx BW (2016) Cytokine production capacity in depression and anxiety. Transl Psychiatry 6:e825.

Wang F, Wang J, An J, Yuan G, Hao X, Zhang Y (2018) Resveratrol ameliorates depressive disorder through the NETRIN1-mediated extracellular signal-regulated kinase/cAMP signal transduction pathway. Mol Med Rep 17:4611–4618.

Wang X, Fu S, Wang Y, Yu P, Hu J, Gu W, Xu XM, Lu P (2007) Interleukin-1beta mediates proliferation and differentiation of multipotent neural precursor cells through the activation of SAPK/JNK pathway. Mol Cell Neurosci 36:343–354.

Wueest S, Item F, Boyle CN, Jirkof P, Cesarovic N, Ellingsgaard H, Böni-Schnetzler M, Timper K, Arras M, Donath MY, Lutz TA, Schoenle EJ, Konrad D (2014) Interleukin-6 contributes to early fasting-induced free fatty acid mobilization in mice. Am J Physiol Regul Integr Comp Physiol 306:R861–R867.

Zonis S, Ljubimov VA, Mahgerefteh M, Pechnick RN, Wawrowsky K, Chesnokova V (2013) p21Cip restrains hippocampal neurogenesis and protects neuronal progenitors from apoptosis during acute systemic inflammation. Hippocampus 23:1383–1394.

Zunszain PA, Anacker C, Cattaneo A, Choudhury S, Musaelyan K, Myint AM, Thuret S, Price J, Pariante CM (2012) Interleukin-1beta: a new regulator of the kynurenine pathway affecting human hippocampal neurogenesis. Neuropsychopharmacol 37:939–949.